Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 223–227
Bioorganic & Medicinal Chemistry Letters

Combinatorial synthesis of anilinoanthraquinone derivatives and evaluation as non-nucleotide-derived $P2Y_2$ receptor antagonists

Stefanie Weyler,[a] Younis Baqi,[a] Petra Hillmann,[a] Marko Kaulich,[a] Andrea M. Hunder,[a,b] Ingrid A. Müller[b] and Christa E. Müller[a,*]

[a]*Pharmaceutical Institute, Pharmaceutical Sciences Bonn (PSB), University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany*
[b]*Fachhochschule Albstadt-Sigmaringen, University of Applied Sciences, Pharmatechnik, Anton-Günther-Str. 51, D-72488 Sigmaringen, Germany*

Received 29 September 2007; revised 23 October 2007; accepted 25 October 2007
Available online 30 October 2007

Abstract—A library of anilinoanthraquinone derivatives was synthesized by parallel Ullmann coupling reaction of bromaminic acid with aniline derivatives in solution using a compact parallel synthesizer. The products were purified by HPLC and evaluated as antagonists at mouse and human $P2Y_2$ receptors. 4-Phenylamino-substituted 1-amino-2-sulfoanthraquinones, for example, 1-amino-4-(2-methoxyphenyl)-2-sulfoanthraquinone (PSB-716), were potent $P2Y_2$ antagonists with IC_{50} values in the low micromolar range.

$P2Y_2$ receptors ($P2Y_2R$) belong to the family of G protein-coupled nucleotide (P2) receptors.[1–4] They are activated by the physiological nucleotides UTP and ATP, and by dinucleotides, such as diadenosine tetraphosphate (Ap_4A).[4] $P2Y_2R$ show a wide distribution in the body, including lung, heart, skeletal muscle, spleen, kidney, and brain.[1–4] There is a lack of potent and selective $P2Y_2R$ antagonists, which are required as pharmacological tools to elucidate the (patho)physiological roles of the receptors.[1–5] In addition, such compounds have potential as novel therapeutics, for example, as anti-inflammatory agents, for the treatment of coronary vasospastic disorders, as analgesics, or as neuroprotective drugs.[1–4,6]

The present study focuses on the development of non-nucleotide-derived $P2Y_2R$ antagonists using Reactive Blue 2 (RB-2, **1**, Fig. 1) as a lead structure. RB-2 is one of the most potent $P2Y_2$ antagonists known to date (IC_{50} 1–5 μM).[4] However, RB-2 also inhibits ectonucleotidases[7] and blocks other P2 receptor subtypes as well.[3,4,8,9] Furthermore, RB-2 has a relatively high molecular weight (MW = 840 g/mol) and bears three negatively charged sulfonate groups. Therefore, it does not exhibit properties that are desirable for a drug (MW < 500 g/mol, not permanently charged at pH 7.4).[10]

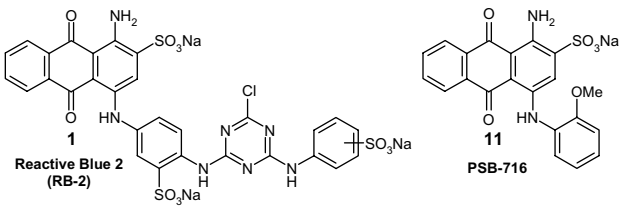


Figure 1. Structures of $P2Y_2$ receptor antagonists Reactive Blue 2 (RB-2) and compound **11** (PSB-716).

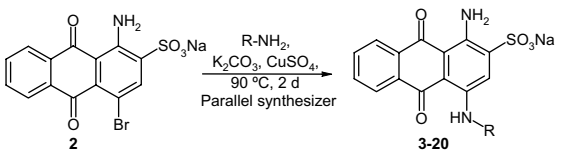
In previous studies anthraquinone derivatives related to RB-2, including Acid Blue 25 (AB-25, compound **3**, Table 2), have been evaluated as P2 receptor antagonists and ectonucleotidase inhibitors.[9,11] The substitution pattern on the aniline ring has been found to be important for activity and P2Y versus P2X receptor selectivity.[11b–d] However, no structure–activity relationships (SARs) have been reported for this class of compounds at $P2Y_2R$.

In the present study, we investigated whether a combinatorial synthetic approach using a compact parallel

Keywords: $P2Y_2$ receptors; Antagonists; Combinatorial synthesis; Anthraquinones; Ullmann coupling reaction.
* Corresponding author. Tel.: +49 228 73 2301; fax: +49 228 73 2567; e-mail: christa.mueller@uni-bonn.de

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.082

Table 1. Yields of anthraquinone derivatives synthesized in a compact parallel synthesizer in solution



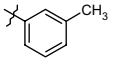
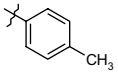
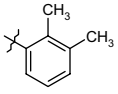
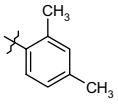
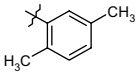
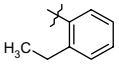
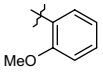
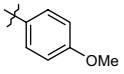
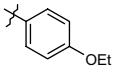
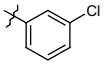
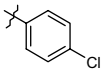
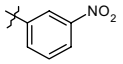
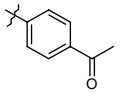
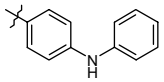
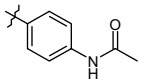
Compound	R	Yield[a]
3		60
4		74
5		83
6		26
7		72
8		42
9		26
10		76
11 (PSB-716)		67
12		82
13		52
15		61
15		16
16		56

Table 1 (*continued*)

Compound	R	Yield[a]
17		35
18		74
19		17
20		90

[a] Isolated yields (purity of products ⩾98%) calculated based on starting compound **2**; true yields were higher since commercial **2** was only 90% pure (main contaminant: desbromo derivative, 8%).[15]

synthesizer for the preparation of a library of 4-phenylamino-substituted 1-amino-2-sulfoanthraquinones would be feasible. Furthermore, we developed an efficient HPLC method for the purification of the products. The compounds were evaluated as $P2Y_2R$ antagonists and SARs were analyzed.

The classical method to obtain the target compounds is the copper-catalyzed Ullmann coupling reaction.[12] Thus, bromaminic acid sodium salt (**2**) was reacted with aniline derivatives in the presence of copper(II) sulfate and sodium carbonate in water at 90 °C for 2 days. These conditions were found to be a good compromise for all the aniline derivatives employed, which possess very different reactivities. Parallel synthesis was performed in polypropylene vials using a MiniBlock™ synthesizer (Mettler Toledo, Switzerland).[13] After lyophilization the products were subjected to purification by HPLC on a Eurospher 100 C 18 column (10 μm, 250 × 20 mm).[14]

Examples for typical chromatograms are shown in Figure 2 for products **7**, **8**, and **13**. The starting compound bromaminic acid (**2**, red) and the side-product 1-amino-4-hydroxy-2-sulfoanthraquinone (**21**, dark red), which results from a substitution of the bromine atom by hydroxide, eluted within the first 5 min. In contrast, the somewhat less polar anilino derivatives **3–20** were eluted between 8 and 14 min thus allowing a baseline separation from the starting compound and the side-product (Fig. 2), which results in very pure products. Purities were determined by LCMS (for details, see Supplementary Information) and found to be >98% in all cases. The products were further characterized by ^{1}H- and ^{13}C NMR spectra, elemental analysis, or high resolution mass spectra, respectively; for a typical example, see footnote.[16]

As shown in Table 1 yields ranged from 16 to 90% depending on the substituents on the phenyl ring. In most cases, yields were greater than 50%; only for 6 products (**6**, **8**, **9**, **15**, **17**, and **19**) lower yields were ob-